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Transport and Metabolism of Alanine and Palmitic Acid by Field-Collected Larvae of *Tedania ignis* (Porifera, Demospongiae): Estimated Consequences of Limited Label Translocation

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Abstract. The epidermis of larvae of *Tedania ignis* (Porifera, Demospongiae) is uniformly ciliated except for the posterior pole. The epidermal cells are long, columnar, and monociliate; each cilium arises from an epidermal crypt; symbiotic bacteria were not observed in larval cells. These lecithotrophic ("nonfeeding") larvae can feed by assimilating dissolved organic materials (DOM) from seawater. Larvae transported both the amino acid alanine (mean = 2.73 pmol larva^{-1} h^{-1}; [S] = 1 μM) and the fatty acid palmitic acid (mean = 16.27 pmol larva^{-1} h^{-1}; [S] = 1 μM) from seawater. Following assimilation, the label from alanine was recovered primarily in small molecular weight compounds; the label from palmitic acid was localized chiefly in the lipid fraction. Estimates of the contribution of transport to metabolism (mean respiration rate = 940.7 pmol O_2 larva^{-1} h^{-1}) reveal that alanine transport is energetically insignificant. Palmitic acid transport, in contrast, could account for 21%–55% of larval metabolism. Autoradiographic analysis of the distribution of the label in larvae suggests that epidermal cells are the chief recipients of the assimilated materials. Thus, the contribution of transport to whole-larva metabolism may underestimate the tissue-specific value. At palmitic acid concentrations of 1 and 0.25 μM, the contribution of transport to the estimated metabolism of the epidermis would be 131% and 33% of energy requirements. Thus, the potential benefits of DOM to larvae are dependent not only on the nature of the epidermal transporters and the solute concentration, but also the degree to which materials are distributed among tissues.

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Introduction

The energy requirements for development of "nonfeeding" (lecithotrophic) larvae of marine invertebrates have historically been thought to be solely derived from the catabolism of maternally provided stores (Chia, 1974; Crisp, 1974; Day and McEdward, 1984). In recent years, however, it has been shown that nonfeeding embryos and larvae can obtain energy from the environment through the transport of dissolved organic materials (DOM) from seawater (Reish and Stephens, 1969; Jaeckle and Manahan, 1989a; Manahan *et al.*, 1989; Welborn and Manahan, 1990; Jaeckle, 1994). Prefeeding embryos of planktotrophic (feeding) larvae can also assimilate organic materials from seawater (*e.g.*, Monroy and Tolis, 1961; Tyler *et al.*, 1966; Epel, 1972; Karp and Weems, 1975; Manahan, 1983a; Schneider and Whitten, 1987).

Analyses of the energetics of larval development indicate that DOM transport by nonfeeding larvae and embryos may be important. The contribution of organic solute transport to metabolic processes can be estimated by comparing joules supplied (through transport) with joules expended (metabolic rate) (Stephens, 1963; Wright, 1981; Manahan *et al.*, 1983; Jaeckle and Manahan, 1989a). These comparisons reveal that the potential energetic benefits of DOM transport vary among both transported compounds and larval forms. In general, for compounds at a concentration of 1 μM, the estimated contribution of transport to the metabolism of nonfeeding larvae and prefeeding embryos ranges from <1% to *ca.* 35% for free amino acids and sugars (Jaeckle and Manahan, 1989a,

1992; Jaeckle, 1994) and from *ca.* 20% to 70% for the fatty acid palmitic acid (Jaeckle, 1994). Thus the potential nutritional and energetic value of DOM in seawater to nonfeeding life history stages of invertebrates is a function of both the quantity and the quality of the organic compounds present and the physiological capacities of the larva.

Most published studies on DOM transport report the capabilities of embryos and larvae of temperate-water species to exploit this potential source of nutriment and energy (see Manahan, 1990, for a recent review). Of these studies, few (Karp and Weems, 1975; DeBurgh and Burke, 1983; Manahan and Crisp, 1983) have examined the spatial distribution of a label (initially associated with the assimilated molecules) within the larval body and how the distribution pattern of the label changes over time. Further, where translocation of materials has been suggested, the larvae used were planktotrophic and the appearance of label in interior cells, *i.e.*, the digestive system, cannot be attributed solely to the assimilatory activity of the ectoderm and subsequent translocation to interior cells *via* a blood-vascular system (*e.g.*, Ruppert and Carle, 1983).

The objectives of this study were to measure transport and metabolism of an amino acid and a fatty acid from seawater, to determine the rates of oxygen consumption, and to follow the distribution of a ^3H label within the larval body (using light-microscopic autoradiography) in field-collected parenchymula larvae of the demosponge *Tedania ignis*. The results of these experiments reveal that for larvae of *T. ignis* the calculated contribution of DOM to whole-larva metabolism is highly dependent on the available solute in solution. Transport and metabolism of palmitic acid ([S] = 1 μM) could account for an average of 37% of the metabolic demand, while <1% of the metabolic rate could be supplied through alanine transport. Following transport, the distribution of the label in larval tissue is not uniform; most of the label was detected in the epidermis after a 2-h continuous exposure to the label. Comparison of the rates of DOM transport to estimates of the metabolic rate of the epidermis reveals that the energetic significance of DOM transport to the epithelium apparently responsible for material assimilation can be very high (>90% compensation of the estimated metabolic rate of the epidermis).

Materials and Methods

Collection and handling of larvae

Larvae of *Tedania ignis* were collected from general plankton samples taken from the Fort Pierce Inlet (*ca.* 27° 28′ N; 80° 18′ W) during April–June of 1991. All samples were collected during flooding tides by deploying a 0.5-m plankton net with 202-μm (mesh size) netting in the tidal flow for 10–15 minutes. Samples were sorted at the Smithsonian Marine Station at Link Port as soon as possible (<1 h) after collection. Larvae of *T. ignis* were placed in 0.2-μm (pore size) filtered seawater (hereafter termed seawater) and held at a temperature of 22.5°C.

Measurement of morphological and physical characteristics

Before the linear dimensions of *Tedania* larvae were measured, individuals were fixed by immersion in 1% OsO_4 in seawater for 1 h, washed in seawater, and then measured (±0.5 μm) using a compound microscope equipped with an ocular micrometer.

For morphological inspection, larvae of *Tedania ignis* were processed in a number of ways. For examination of surface structures, larvae were fixed in 1% OsO_4 in seawater for 1 h, washed with seawater, dehydrated with an ascending ethanol series, and critical-point dried using CO_2 as the transition fluid. The specimens were mounted on stubs, coated with a gold-palladium mixture, and examined using a Novascan 30 scanning electron microscope. For light microscopic histology and autoradiography and transmission electron microscopy, specimens were initially fixed in 2.5% glutaraldehyde in seawater and then post-fixed in 2% OsO_4 in a 1.25% solution of $NaHCO_3$. This material was dehydrated using ethanol, transferred into propylene oxide, and embedded in an epoxy resin (Epon 812). Thick sections (*ca.* 1 μm) were cut with a glass knife, stained with "Richardson's stain" (Richardson *et al.*, 1960) and examined with a compound microscope. Thin sections (*ca.* 60 nm) were cut with a diamond knife, stained with saturated aqueous solutions of lead citrate and of uranyl acetate, and examined with a Zeiss EM-9S transmission electron microscope.

For determinations of larval organic weight (biomass), larvae were processed using the procedures described in Jaeckle and Manahan (1989b).

Measurement of oxygen consumption

The respiration rate of *Tedania ignis* larvae was measured following the procedures outlined in Jaeckle (1994) at a temperature of 22.5 ± 0.05°C. All measured respiration values were corrected for the self-consumption rate of the electrode (<9% of the larval respiration rate). The rate of oxygen consumption (mol O_2 $larva^{-1}$ h^{-1}) was calculated as the slope of a regression line of the collected data, divided by the number of larvae, and multiplied by 60 min/h. The measured rates of oxygen consumption were converted to the energy units by using an oxyenthalpic equivalent of 480 kJ mol O_2^{-1} (the average oxyenthalpic equivalent for protein [527 kJ mol O_2^{-1}], lipid [441 kJ mol O_2^{-1}], and carbohydrate [473 kJ mol O_2^{-1}], all from Gnaiger [1983]).

Alanine and palmitic acid transport

Larvae were transferred to 10 ml of seawater in an autoclave-sterilized 20-ml scintillation vial (for experiments with palmitic acid, the vial was previously silanized with Silvue [SDS Coatings, Inc.]). All transport was measured at larval concentration of ≤4 larvae/ml and an added solute concentration of 1 μM. After the addition of the label (^{3}H-alanine or ^{3}H-palmitic acid, New England Nuclear, specific activities 70 or 84 Ci/mmol and 60 Ci/mmol, respectively) and cold carrier, the vial was mixed by inversion and the first sample removed. Each sample of larvae (≤5 larvae per sample) was treated following the methods described in Jaeckle and Manahan (1989a). For one experiment, the rate of alanine transport was measured as the accumulation of radioactivity in larvae after 1 h of continuous exposure to the label. For all other experiments, the measured amount of radioactivity per larva (corrected for signal quenching) was converted to moles of material per individual, and the rate of transport was calculated as the slope of a regression line describing the relationship between moles of material per larva and time.

Alanine and palmitic acid metabolism

Larvae remaining after the 1-h incubation in the solution of either ^{3}H-alanine or palmitic acid (see above) were removed and pipetted onto a glass-fiber filter. The sample of larvae was gently washed twice with 20 ml each of cold (5°C) seawater, and the number of individuals on the filter was counted. After washing, the filters were then placed into a −70°C freezer to stop all metabolic activity of the larvae. The sample of larvae was then lyophilized for 8 h (<10 μm Hg), 5 ml of distilled water was added, and the larval tissue was homogenized using an ultrasonic tissue disrupter (Fisher model #300). Samples of the tissue homogenate were separated into general biochemical fractions (protein, lipid, and small molecular weight compounds) using the methods described in Jaeckle and Manahan (1989b). Each resulting fraction and a sample of the intact homogenate were dissolved in tissue solubilizer, and the radioactivity in each sample was measured 48 h after the addition of scintillation cocktail. The measured amount of radioactivity per fraction (corrected for signal quenching) was converted to a percentage of the total by dividing the radioactivity in each fraction by the amount of radioactivity in the sample of tissue homogenate.

Localization of the label following assimilation within larvae

Light-microscopic autoradiography was used to determine the location of the ^{3}H-label in larvae. Larvae were continuously exposed to radiolabeled alanine and palmitic acid (each at 1 μM added concentration) for 10, 60, or 120 min. At the end of each exposure, the larvae were washed twice with seawater (10 ml each time) and fixed and processed as described above. Serial thick sections were cut, then secured onto acid-cleaned microscope slides. The slides were immersed into a liquid photographic emulsion (Ilford #Kd.5), air dried for 24 h, and stored in a light-tight box at 5°C. The slides were developed according to manufacturer specifications, and the autoradiograms were examined and photographed with a compound microscope.

Results

Physical characteristics

Field-collected parenchymula larvae of *Tedania ignis* are orange-red in coloration and averaged 818.5 ± 17.5 μm in length and 576.3 ± 17.7 μm in width (both mean ± 1 standard error (SE); n = 16 larvae). The average length:width ratio for these larvae was 1.4 ± 0.1 (mean ± 1 SE, n = 16 larvae). With the sole exception of the posterior pole (assigned as the trailing pole during swimming), the larvae were uniformly ciliated (Fig. 1). The epidermis is composed primarily of long, thin, monociliate cells (Figs. 2, 3); each cilium emerges from the cell body through an epidermal crypt or pit (Fig. 3). This morphological examination of the epidermis did not reveal either intra- or extracellular bacteria (not shown); hence the measured rates of solute transport (below) represent the physiological activity of larval cells alone.

The average weight of a *Tedania* parenchymula larva was 16.99 ± 0.72 μg/larva (mean ± 1 SE, n = 14 groups of larvae @ <7 larvae/group).

Alanine and palmitic acid transport

Both alanine and palmitic acid were transported from seawater by larvae of *Tedania ignis*, but the rates of transport differed between the two compounds. Alanine was transported at rates that averaged 2.73 ± 0.6 pmol alanine larva^{-1} h^{-1} (mean ± 1 SE, n = 3 experiments). The rates of palmitic acid transport were nearly 6× higher and averaged 16.27 ± 2.3 pmol palmitic acid larva^{-1} h^{-1} (mean ± 1 SE, n = 4 experiments). The fate of the radioactive label in larval tissue also differed between the two compounds. For larvae exposed to ^{3}H-alanine, most of the label (64%) was recovered in the small molecular weight compound fraction, *e.g.*, soluble in cold 5% trichloroacetic acid (TCA). The remaining label was found in the TCA-insoluble (macromolecular) fraction (23%) and in lipoic materials (14%) localized in the $CHCl_3$-soluble fraction. For larvae exposed to ^{3}H-palmitic acid, most of the material was recovered in the $CHCl_3$-soluble fraction (79%), and the remaining radioactivity was divided between the

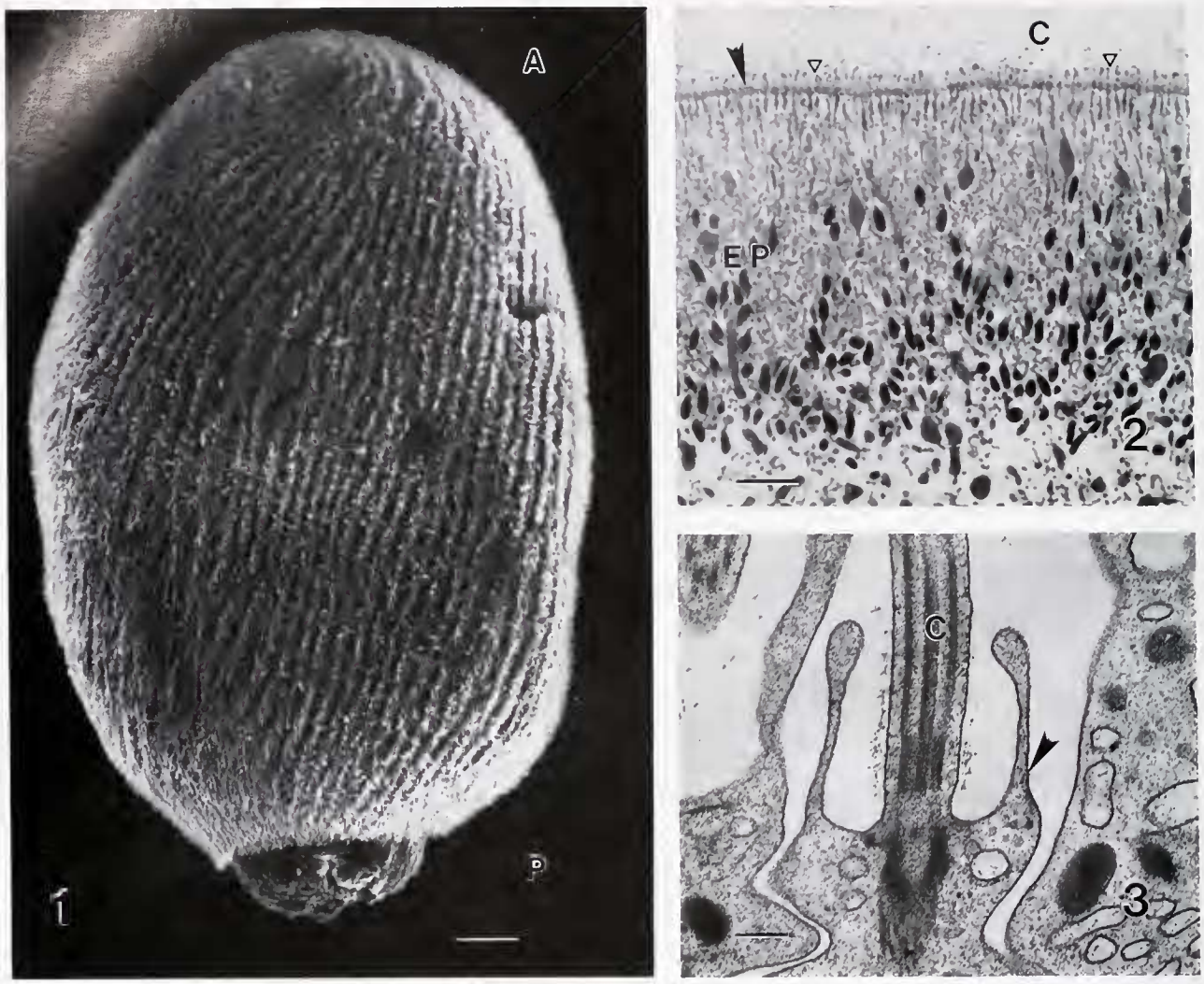

Figure 1. Scanning electron micrograph of a lateral view of a field-collected larva of *Tedania ignis*. The anterior (A) and posterior (P) regions of the larvae were designated as the leading and trailing poles during swimming. Scale bar = 50 μm.

Figures 2 and 3. Light and transmission electron micrographs of the epidermis of parenchymulae of *Tedania ignis*.

Figure 2. Light micrograph of a larva of *Tedania ignis*. The epidermis (EP) is composed primarily of thin, columnar, ciliated cells. Subapically a continuous line (large arrowhead) is present and is suggestive of intercellular junctional complexes. The presence of epidermal crypts from which the cilia (C) arise at the apices of the ciliated cells is denoted by the small arrowheads. Scale bar = 10 μm.

Figure 3. Transmission electron micrograph of the apical region of a ciliated epidermal cell of a larva of *Tedania ignis*. A single cilium (C) can be seen emerging from the epidermal crypt of the epidermal cell. Scale bar = 0.25 μm.

TCA-soluble fraction (14%) and the TCA-insoluble fraction (8%).

Larval respiration

The respiration rate (O_2 consumption) of larvae of *Tedania ignis* was variable among the groups of larvae examined. Values ranged from 846.8 to 1113.9 pmol O_2 $larva^{-1}$ h^{-1}, with an average of 940.7 ± 70.0 pmol O_2 $larva^{-1}$ h^{-1} (mean ± 1 SE, n = 5 independent collections of larvae).

Autoradiographic analysis of the distribution of the ^3H-label

The biochemical nature (macromolecular or small molecular weight) of the molecules containing the label

cannot be ascertained by light-microscopic autoradiography of glutaraldehyde-fixed material. Glutaraldehyde is a good preservative of cellular details, in part because it acts by cross-linking primary amines. This activity may result in a false intracellular localization of the label, if the label-bearing molecule resides in the extracellular space (*e.g.*, Peters and Ashley, 1967). However, the autoradiographic analysis presented here was designed to ascertain whether the label, after transport, was distributed throughout the entire larval body, not to determine the pathway of material movement (paracellular or transcellular).

After 10 min of exposure to either ^{3}H-alanine or palmitic acid, the label is found in or around the cells of the epidermis (Fig. 4A), as evidenced in the autoradiograms by the appearance of silver granules overlying these cells. Even though larvae were continuously exposed to the label for up to 2 h, examination of the autoradiograms (Fig. 4A–C) indicates that most of the label remained associated with the cells of the epidermis.

Discussion

Most research on the larvae of demosponges has focused on their morphological or behavioral characters (*e.g.*, Berquist *et al.*, 1970; Woollacott, 1990, 1993; Kaye and Reiswig, 1991). The morphology of the epidermis of larvae of *Tedania ignis* (subclass Poecilosclerida) closely approximates that described for larvae of the haplosclerid demosponge *Haliclona tubifera* (Woollacott, 1993). For both species, the epidermis is composed chiefly of elongate columnar cells (each with a single cilium arising from an epidermal crypt). The posterior pole is aciliate in both, but the enlarged ciliary band that exists at the intersection of the lateral and posterior surfaces in *H. tubifera* larvae is wanting in larvae of *T. ignis*. The physiological significance of the epidermal crypts remains unknown, but these depressions in the larval epidermis do increase the apical surface area of the cells and represent a potential morphological correlate to solute transport (Oschman, 1978).

Although parenchymula larvae of *Tedania ignis* lack a functional digestive system, these larvae have the physiological capacity to acquire nutrients and energy from their environment through the transport of DOM from seawater. A comparison of the energy acquired through transport with the metabolic rate indicates that the potential energetic importance of alanine and palmitic acid transport differs (Table 1). The energy supplied through

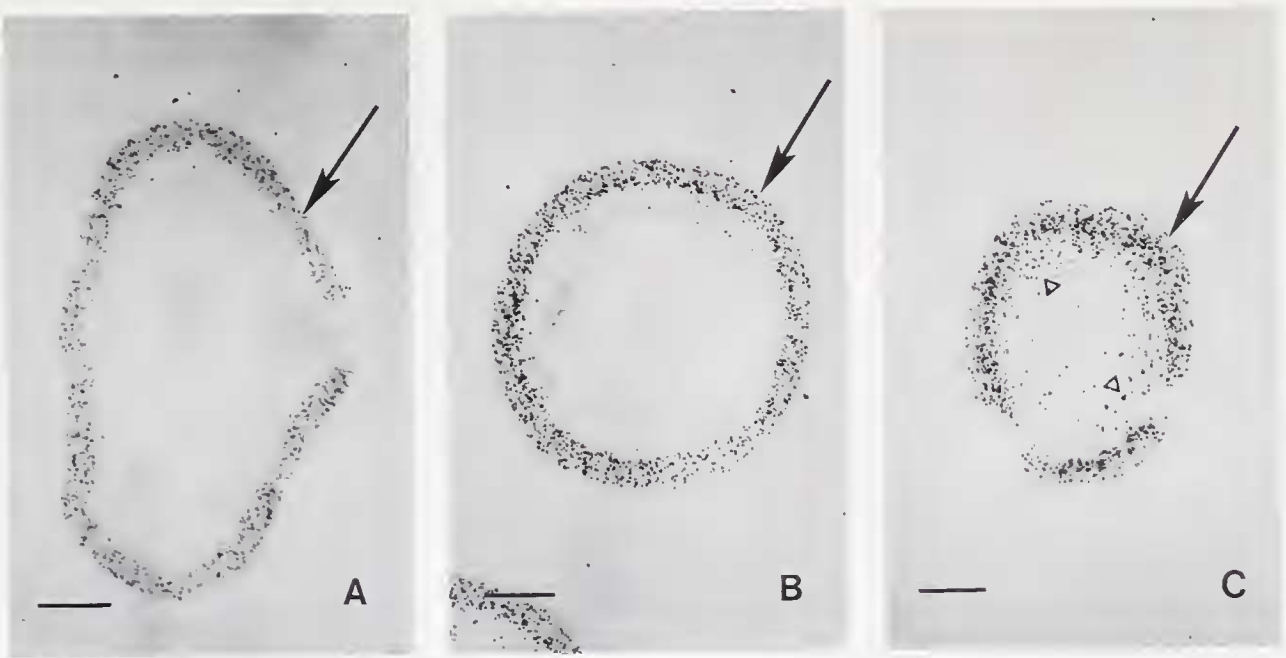

Figure 4. Light microscopic audioradiograms depicting the distribution of the radioactive label in transverse sections of larvae of *Tedania ignis* that were exposed to ^{3}H-palmitic acid for different periods of time. (A) After 10 min of exposure to ^{3}H-palmitic acid, the presence of latent images (reduced silver grains) overlies only the cells of the epidermis (arrow). Scale bar = 303 μm. (B) After a 1-h exposure, the number of latent images over the epidermis has increased, but no label is detectable over other regions of the section. Scale bar = 303 μm. (C) After 2 h of continuous exposure to ^{3}H-palmitic acid, the latent images are more intense and the label primarily overlies the epidermis, but there is evidence for the presence of radioactive materials in interior regions of the larva. Scale bar = 303 μm.

Table I

Comparison of the potential contribution of palmitic acid and alanine transport ([S] = 1 μM) to the energy metabolism of Tedania ignis *larvae*

Solute	Transport[1]	O_2 demand[2]	Metabolism[3]	% Compensation[4]	[S] for 100% compensation[5]
Palmitic acid	13.19	317.17	940.66	33.7	3.0
	22.54	518.42	940.66	55.1	1.8
	11.86	272.78	940.66	20.9	3.4
	16.27	374.30	940.66	39.8	2.5
avg.	16.27	370.67		37.4	2.7
s.d.	4.66	106.91		14.2	0.7
Alanine	1.77	5.31	940.66	0.6	166.7
	3.87	11.61	940.66	1.2	83.3
	2.53	7.59	940.66	0.8	125.0
avg.	2.72	8.17		0.6	166.7
s.d.	1.06	3.19		0.3	41.7

[1] Transport rate = pmol × $larva^{-1}$ h^{-1}.

[2] O_2 demand = the transport rate × the mol O_2 required for complete catabolism of palmitic acid (23 mol O_2/mol Pal) and alanine (3 mol O_2/mol Ala).

[3] Metabolism = the average respiration rate per larva (pmol O_2 × $larva^{-1}$ h^{-1}).

[4] % Compensation = the oxygen demand ÷ the metabolic rate × 100.

[5] [S] for complete compensation = 100 ÷ % compensation of the metabolic rate.

alanine transport ([S] = 1 μM) could supply <1% of metabolic demands, and complete compensation of the metabolic rate from alanine transport would require ecologically unrealistic alanine concentrations for seawater samples (range: 83–167 μM). In contrast, the energy contribution supplied through palmitic acid transport ([S] = 1 μM) may be quantitatively important. The average transport rate of palmitic acid is nearly 6× that found for alanine transport and, owing to the difference in energy content of the two compounds, the energy acquired through palmitic acid transport could supply between 21% and 55% (mean ± 1 SE = 37.4% ± 7.1; Table I) of the metabolic demand. A palmitic acid concentration ranging from 2 to 3 μM would be necessary if all of the energy requirements of *Tedania* larvae were supplied solely through the transport and catabolism of this compound.

Following transport of both alanine and palmitic acid, the ^{3}H-label was recovered in each of the measured biochemical fractions (protein, lipids, and small molecular weight compounds). Because a ^{3}H-label was used in the experiments, the pathways that place the label in each of the three fractions remain unresolved. Yet a comparison of results of experiments using either ^{3}H-alanine or palmitic acid revealed a differential distribution of the label among the biochemical fractions. After alanine transport, most of the label was recovered in the small molecular weight fraction followed, in sequence, by the macromolecular and lipid fractions. This pattern of label distribution following alanine transport is consistent with previously published accounts in which ^{14}C served as the radioactive label (Manahan, 1983b; Jaeckle and Manahan, 1989a,c). The distribution of label recovered in larvae exposed to ^{3}H-palmitic acid was different; most of the label was found in the lipid fraction, with the remainder being localized in the small molecular weight compounds and macromolecule fractions. This pattern of label distribution compares well with that described for adult *Stauronereis rudolphi* (Annelida: Polychaeta) after exposure to ^{14}C-1-palmitic acid (Testerman, 1972).

The concentrations of both free amino acids and free fatty acids in seawater vary from below detection (< fM) to low μM levels (Testerman, 1972; Bunde and Fried, 1978; Mopper and Lindroth, 1982; Carlucci *et al.*, 1984; Fuhrman and Bell, 1985; Laanbroek *et al.*, 1985). Thus the estimates of energetic contribution presented above are dependent upon the physiological state of the larvae and the concentration of the organic materials in seawater. For larvae of *Tedania ignis*, even when exposed to high concentrations of amino acids in surface waters (μM), the net energetic benefit is likely to be small. The concentration of free fatty acids in subtropical Floridian waters was reported to be 50–80 $\mu g\ l^{-1}$, a range that is equivalent to about 0.25 μM palmitic acid (Bunde and Fried, 1978). At this concentration, assuming that the K_+ of the palmitic acid transporter is greater than 1.0 μM, the energetic contribution of transport to the larva would be, on average, 9.4% of the metabolic demand.

Attempts to quantify the energetic importance of the transport of organic solutes from seawater usually involve a comparison of the energy gained (through transport) to the metabolic rate. Material assimilation, however, is a regional process restricted to the outer epithelium of lecithotrophic larvae and, in contrast, metabolic rate sums over the metabolic activities of all cells. Comparison of

energy supply (transport) and demand (O_2 consumption) for lecithotrophic larvae is based on the assumption that the transported organic solutes are distributed throughout the entire larval body. For larvae of *Tedania ignis*, the assumption of translocation to parenchymal tissues after assimilation by the outer epithelium does not seem to be true. Examinations of autoradiograms of larvae exposed to radiolabeled alanine and palmitic acid for up to 2 h reveals that nearly all the label remains with the cells putatively responsible for transport.

The suggestion that DOM transport may be a regionally important source of nutrition and energy is not new. Earlier researchers (*e.g.*, Pequignat, 1966; Ferguson, 1967, 1970) working on DOM transport in adult invertebrates suggested that the epidermis may be the sole recipient of the assimilated materials and that there is little translocation of materials from the adult endoderm to the ectoderm. Later studies (*e.g.*, Pearse and Pearse, 1973; Pequignat, 1973; Ferguson, 1980; Chien and Rice, 1985; Rice and Stephens, 1987) indicated that there could be translocation of material to interior cells and that the viability of the epidermis was not dependent upon exogenously supplied nutrients. In his review of integumentary transport by invertebrates, Wright (1988) reported that "the nutritional impact of DOM uptake may vary with the specific integumental site of transport: over much of the surface of the integument, accumulated substrates will support the nutritional needs of those cells; uptake into other integumental regions may result in a rapid transcellular movement of accumulated materials to the hemolymph for transport to deeper tissues. To the extent that this type of specialized 'partitioning' of accumulated DOM occurs in the integument, the calculation of the nutritional potential that have been described here will, of course, under- or overestimate the nutritional potential of uptake for a given organism or tissue."

Table II

The contribution of palmitic acid and alanine transport to the metabolism of the larval epidermis at substrate concentrations of 1 μM and 0.25 μM

Solute	O_2 demand (1 μM, 0.25 μM)[1]	% Compensation (1 μM, 0.25 μM)[2]
Palmitic acid	370.7, 92.7	131%, 33%
Alanine	8.2, 2.0	3%, <1%

The estimated metabolic rate of the epidermis (282.2 pmol O_2 × epidermis^{-1} h^{-1}) is calculated as the estimated weight fraction of the epidermis (0.3) times the average metabolic rate of the intact larvae (940.7 pmol O_2 × larva^{-1} h^{-1}).

[1] O_2 demand ([S] = 1 μM) = the transport rate × the mol O_2 required for complete catabolism of palmitic acid (23 mol O_2/mol Pal) and alanine (3 mol O_2/mol Ala). O_2 demand ([S] = 0.25 μM) is calculated by dividing the O_2 demand (1 μM) by 4 (Pal and Ala transport is assumed to be first-order at substrate concentrations of 1 μM or lower).

[2] % Compensation of the metabolic rate of the epidermis is calculated by dividing the O_2 demand$_{(epidermis)}$ by the estimated metabolic rate$_{(epidermis)}$ and multiplying by 100.

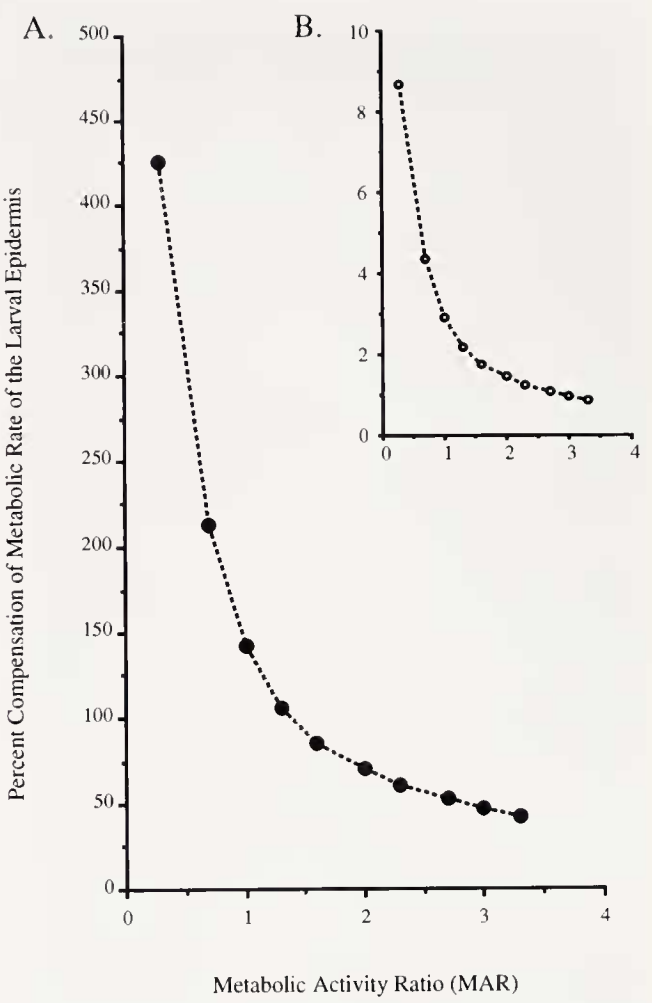

Figure 5. The change in the estimated contribution of palmitic acid (A) and alanine (B) transport ([S] = 1 μM each) from seawater to the metabolism of the epidermis of larvae of *Tedania ignis*. The range in metabolic activity ratios (MARs) represents the relative difference in the metabolic activities of epidermal cells and internal cells.

The estimated contributions of DOM transport to the metabolism of *Tedania ignis* larvae are detailed in Table I. If, however, most of the assimilated DOM from seawater is metabolized solely by the cells responsible for transport, then evaluations of the energetic importance of this form of nutrient acquisition underestimate the potential *epidermis*-specific value. In larvae of *T. ignis*, assimilated materials apparently remain within the epidermis; to estimate the importance of transport to the metabolism of the epidermis of *Tedania* larvae, the following analysis was done. Cross-sections (1 μm thick) of *Tedania* larvae were photographed and the negatives printed. From the

photographs, the cross-sections of three larvae were cut out and weighed on an analytical balance. The area of the section representing the epidermis was then cut out and the remaining photographic images of the internal cells were reweighed. If it is assumed that larvae of *T. ignis* are cylindrical and that all cells have the same weight density, then the areal proportion of the epidermis in the photograph is equal to the proportion of a larva's organic weight that is represented by the cells. On the basis of this analysis, the epidermis of *T. ignis* larvae represents, on average, 30% of the total larval biomass (*ca.* 5 μg). If all cells of a larva have the same respiration rate, then the metabolic rate of the epidermis is 282.2 pmol O_2 $larva^{-1}$ h^{-1} (0.3 · 940.7 O_2 $larva^{-1}$ h^{-1}). The energetic contribution of palmitic acid transport ([S] = 1 μM and 0.25 μM) to the metabolism of the larval epidermis is potentially important (Table II). It seems unlikely, however, that the metabolic rate is constant among all cells, given the morphological differences among cell types in sponge larvae (Woollacott, 1990, 1993). The theoretical contribution from transport to metabolism can be adjusted to account for the differences in cellular metabolism. Changes in the potential contribution of alanine and palmitic acid transport to metabolism as a function of the relative activities of the external and internal cells are shown in Figure 5. In this figure, a metabolic activity ratio (MAR) of 1 represents the condition in which the metabolic rate of the epidermal cells is directly proportional to their weight-fraction of larval tissue; *i.e.*, all cells respire at the same weight-specific rate. A metabolic activity ratio of 2 equals the condition in which the metabolic rate of the epidermal cells is twice that of their interior counterparts. Complete compensation of the estimated metabolic demand of the epidermis would be provided through palmitic acid transport ([S] = 1 μM) at a MAR < 1.3. For alanine transport alone, 100% compensation of the metabolic rate could not be accomplished.

Sponge parenchymula larvae can live in plankton for periods of time lasting from hours to days (*e.g.*, Berquist *et al.*, 1970; Woollacott, 1990, 1993; Kaye and Reiswig, 1991). During their planktonic existence they are functionally incapable of ingesting particulate forms of food. Despite this inadequacy, these lecithotrophic larvae are physiologically capable of assimilating DOM from seawater across their epidermis; hence they are not nutritionally independent of their environment. The potential benefits obtained through DOM transport and metabolism are not, however, dependent only on the substrate concentration and the class and species of the organic materials present in seawater, but also on whether the assimilated materials are distributed among all larval cells.

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Literature Cited

Berquist, P. R., M. E. Sinclair, and J. Hogg. 1970. Adaptation to intertidal existence: Reproductive cycles and larval behavior in Demospongiae. *Symp. Zool. Soc. Lond.* **25:** 247–271.

Bunde, T. A., and M. Fried. 1978. The uptake of dissolved free fatty acids from seawater by a marine filter feeder *Crassostrea virginica*. *Comp. Biochem. Physiol.* **60A:** 139–144.

Carlucci, A. F., D. B. Craven, and S. M. Hendrichs. 1984. Diel production of dissolved free amino acids in waters off southern California. *Appl. Environ. Microbiol.* **48:** 165–170.

Chia, F. S. 1974. Classification and adaptive significance of developmental patterns in marine invertebrates. *Thalassia Jugosl.* **10:** 121–130.

Chien, P. K., and M. A. Rice. 1985. Autoradiographic localization of exogenously supplied amino acids after uptake by the polychaete *Glycera dibranchiata* Ehlers. *Wasmann. J. Biol.* **43:** 60–71.

Crisp, D. J. 1974. Energy relations of marine invertebrate larvae. *Thalassia Jugosl.* **10:** 103–120.

Day, R., and L. McEdward. 1984. Aspects of the physiology and ecology of pelagic larvae of marine benthic invertebrates. Pp. 93–120 in *Marine Plankton Life Cycle Strategies*, K. A. Steidinger and L. A. Walker, eds. CRC Press, Inc., Boca Raton, Florida.

DeBurgh, M. E., and R. D. Burke. 1983. Uptake of dissolved amino acids by embryos and larvae of *Dendraster excentricus* Eschscholtz (Echinodermata: Echinoidea). *Can. J. Zool.* **61:** 349–354.

Epel, D. 1972. Activation of an Na^+-dependent amino acid transport system upon fertilization of sea urchin eggs. *Exp. Cell Res.* **72:** 74–89.

Ferguson, J. C. 1967. An autoradiographic study of the utilization of free amino acids by starfishes. *Biol. Bull.* **133:** 317–329.

Ferguson, J. C. 1970. An autoradiographic study of the translocation and utilization of amino acids by starfishes. *Biol. Bull.* **141:** 122–129.

Ferguson, J. C. 1980. The non-dependency of a starfish on epidermal uptake of dissolved organic matter. *Comp. Biochem. Physiol.* **66A:** 461–465.

Fuhrman, J. A., and T. M. Bell. 1985. Biological considerations in the measurement of dissolved free amino acids in seawater and implications for chemical and biological studies. *Mar. Ecol. Prog. Ser.* **25:** 13–21.

Gnaiger, E. 1983. Calculations and energetic and biochemical equivalents of respiratory oxygen consumption. Pp. 337–345 in *Polarographic Oxygen Sensors*, E. Gnaiger and H. Forstner, ed. Springer-Verlag, Berlin.

Jaeckle, W. B. 1994. Rates of energy consumption and acquisition by lecithotrophic larvae of *Bugula neritina* (Bryozoa: Cheilostomata). *Mar. Biol.* **119:** 517–523.

Jaeckle, W. B., and D. T. Manahan. 1989a. Feeding by a "nonfeeding" larva: uptake of dissolved amino acids from seawater by lecithotrophic larvae of the gastropod *Haliotis rufescens*. *Mar. Biol.* **103:** 87–94.

Jaeckle, W. B., and D. T. Manahan. 1989b. Growth and energy imbalance during the development of a lecithotrophic molluscan larva *Haliotis rufescens*. *Biol. Bull.* **177:** 237–246.

Jaeckle, W. B., and D. T. Manahan. 1989c. Amino acid uptake and metabolism by larvae of the marine worm *Urechis caupo* (Echiura), a new species in axenic culture. *Biol. Bull.* **176:** 317–326.

Jaeckle, W. B., and D. T. Manahan. 1992. Experimental manipulations of the organic chemistry of seawater: implications for studies of energy budgets in marine invertebrate larvae (Mollusca, Echinodermata). *J. Exp. Mar. Biol. Ecol.* **156:** 273–284.

Karp, G. C., and M. D. Weems. 1975. ^{3}H-amino acid uptake and incorporation in sea urchin gastrulae and exogastrulae: an autoradiographic study. *J. Exp. Zool.* **194:** 535–546.

Kaye, H. R., and H. M. Reiswig. 1991. Sexual reproduction in four Caribbean commercial sponges. III. Larval behavior, settlement, and metamorphosis. *Invertebr. Reprod. Dev.* **19:** 25–35.

Laanbroek, H. J., J. C. Verplanke, K de Visscher, and R. De Vuyst. 1985. Distribution of phyto- and bacterioplankton growth and biomass parameters, dissolved inorganic nutrients and free amino acids during a spring bloom in Oosterschelde basin, the Netherlands. *Mar. Ecol. Prog. Ser.* **25:** 1–11.

Manahan, D. T. 1983a. The uptake of dissolved glycine following fertilization of oyster eggs, *Crassostrea gigas* (Thunderg). *J. Exp. Mar. Biol. Ecol.* **68:** 53–58.

Manahan, D. T. 1983b. The uptake and metabolism of dissolved amino acids by bivalve larvae. *Biol. Bull.* **164:** 236–250.

Manahan, D. T. 1990. Adaptations by invertebrate larvae for nutrient acquisition from seawater. *Am. Zool.* **30:** 147–160.

Manahan, D. T., and D. J. Crisp. 1983. Autoradiographic studies on the uptake of dissolved amino acids from seawater by bivalve larvae. *J. Mar. Biol. Ass. U.K.* **63:** 673–682.

Manahan, D. T., J. P. Davis, and G. C. Stephens. 1983. Bacteria-free sea urchin larvae: selective uptake of neutral amino acids from seawater. *Science* **220:** 204–206.

Manahan, D. T., W. B. Jaeckle, and S. D. Nourizadeh. 1989. Ontogenic changes in the rates of amino acid transport from seawater by marine invertebrate larvae (Echinodermata, Echiura, Mollusca). *Biol. Bull.* **176:** 161–168.

Monroy, A., and H. Tolis. 1961. Uptake of radioactive glucose and amino acids and their utilization for incorporation into proteins during maturation and fertilization of the eggs of *Asterias forbesii* and *Spisula solidissima*. *Biol. Bull.* **147:** 456–460.

Mopper, K., and P. Lindroth. 1982. Diel and depth variations in dissolved free amino acids and ammonium in the Baltic Sea determined by shipboard HPLC analysis. *Limnol. Oceanogr.* **27:** 336–347.

Oschman, J. L. 1978. Morphological correlates of transport. Pp. 55–92 in *Membrane Transport in Biology*, vol. 3, G. Giebisch, D. C. Tosteson, and H. H. Ussing, eds. Springer-Verlag, Berlin.

Pearse, J. S., and V. B. Pearse. 1973. Removal of glycine from solution by the sea urchin *Strongylocentrotus purpuratus*. *Mar. Biol.* **19:** 281–284.

Pequignat, E. 1966. 'Skin digestion' and epidermal absorption in irregular and regular urchins and their probable relation to the outflow of spherule-coelomocytes. *Nature* **210:** 397–399.

Pequignat, E. 1973. A kinetic and autoradiographic study of the direct assimilation of amino acids and glucose by organs of the mussel *Mytilus edulis*. *Mar. Biol.* **19:** 227–244.

Peters, T., and C. A. Ashley. 1967. An artefact in radioautography due to binding of free amino acids to tissues by fixatives. *J. Cell Biol.* **33:** 53–60.

Reish, D. J., and G. C. Stephens. 1969. Uptake of organic materials by aquatic invertebrates. V. The influence of age on the uptake of glycine-C14 by the polychaete *Neanthes arenaceodentata*. *Mar. Biol.* **3:** 352–355.

Rice, M. A., and G. C. Stephens. 1987. Uptake and internal distribution of exogenously supplied amino acids in the Pacific oyster, *Crassostrea gigas* (Thunberg). *Aquaculture* **66:** 19–31.

Richardson, K. C., L. Jarret, and H. E. Finke. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Tech.* **35:** 313–323.

Ruppert, E. E., and K. J. Carle. 1983. Morphology of metazoan circulatory systems. *Zoomorphology* **103:** 193–208.

Schneider, E. G., and D. J. Whitten. 1987. Uptake and metabolism of nucleosides by embryos of the sea urchin *Strongylocentrotus purpuratus*. *Exp. Cell. Res.* **168:** 1–14.

Stephens, G. C. 1963. Uptake of organic materials by aquatic invertebrates. II. Accumulation of amino acids by the bamboo worm, *Clymenella torquata*. *Comp. Biochem. Physiol.* **10:** 191–202.

Testerman, J. K. 1972. Accumulation of free fatty acids from seawater by marine invertebrates. *Biol. Bull.* **142:** 160–177.

Tyler, A., J. Piatigorsky, and H. Ozaki. 1966. Influence of individual amino acids on uptake and incorporation of valine, glutamic acid, and arginine by unfertilized and fertilized sea urchin eggs. *Biol. Bull.* **131:** 204–217.

Welborn, J. R., and D. T. Manahan. 1990. Direct measurement of sugar uptake from seawater into molluscan larvae. *Mar. Ecol. Prog. Ser.* **65:** 233–239.

Woollacott, R. M. 1990. Structure and swimming behavior of the larva of *Halichondria melanodocia*. (Porifera: Demospongiae). *J. Morph.* **205:** 135–145.

Woollacott, R. M. 1993. Structure and swimming behavior of the larva of *Haliclona tubifera* (Porifera: Demospongiae). *J. Morph.* **218:** 301–321.

Wright, S. H. 1981. A nutritional role for amino acid transport in filter-feeding marine invertebrates. *Am. Zool.* **22:** 621–634.

Wright, S. H. 1988. Nutrient transport across the integument of marine invertebrates. Pp. 173–218 in *Advances in Environmental and Comparative Physiology*, Vol. 2, R. Gilles, ed. Springer-Verlag, Berlin.